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Original article

Plasma norepinephrine is an independent predictor of adverse cerebral and cardiovascular events in type 2 diabetic patients without structural heart disease

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ABSTRACT

Background: Resting plasma norepinephrine (NE) level was reportedly related to high mortality in patients with heart failure. The current study investigated whether resting NE could predict long-term major adverse cerebral and cardiovascular events (MACCEs) in Japanese type 2 diabetic patients without heart disease.
Methods and subjects: We evaluated resting NE in 95 patients with type 2 diabetes who did not have severe complications. Based on the ROC curves, high NE was defined as ≥333 pg/ml. Accurate follow-up information during a mean of 3.6 ± 1.9 years was obtained in 27 high NE patients (13 female, mean age 64 ± 12 years) and 68 low NE patients (29 female, 60 ± 12 years).
Essential results: The Kaplan–Meier curves revealed that MACCE-free ratio was significantly lower in the high NE patients than in the low NE patients (log-rank 10.3, $p = 0.0013$). Cox proportional hazards regression analysis revealed that female gender (hazard ratio 7.75), low baroreflex sensitivity (hazard ratio 6.66), and high NE (hazard ratio 5.40) were independently associated with the incidence of MACCE.
Principal conclusions: Our results suggest that resting NE is comparably useful to identify the high-risk patients for MACCE to baroreflex sensitivity in type 2 diabetic patients. The results also suggest that pathogenic sympathetic activation leading to MACCE may be identified by the assessment of resting NE, more easily and less expensively compared to cardiac iodine 123 metaiodobenzylguanidine scintigraphy in this population.

Introduction

The autonomic imbalance, characterized by increased sympathetic activity and/or decreased parasympathetic activity, is tightly related to cardiovascular mortality. In our series of clinical studies investigating baroreflex sensitivity (BRS) in patients with type 2 diabetes mellitus without structural heart disease [1–5], the presence of essential hypertension [1], microalbuminuria [2], high levels of high-sensitivity C-reactive protein [3], brain natriuretic peptide [4], hypoadiponectinemia [5], or major adverse cerebral and cardiovascular events (MACCEs) [6] have been associated with low levels of BRS. On the other hand, cardiac adrenergic nerve activity has been estimated by use of iodine 123 metaiodobenzylguanidine (^{123}I-MIBG) as a noradrenaline analog [7,8]. In the previous study, we demonstrated that the abnormally increased washout ratio (WR) of cardiac ^{123}I-MIBG at baseline has long-term cardiovascular predictive value in Japanese patients with type 2 diabetes without structural heart disease [9].

A previous Studies of Left Ventricular Dysfunction (SOLVD) trial indicated that patients with asymptomatic left ventricular dysfunction had early increases in plasma norepinephrine (NE), renin activity, and atrial natriuretic peptide compared with healthy control subjects [10]. There is consistent evidence that high sympathetic tone, as measured by plasma NE, predicts mortality in chronic heart failure [10,11], whereas in myocardial infarction, this neurohormone is a weak predictor of adverse outcomes [12]. The objective of this study is to clarify the predictive value of resting plasma NE for MACCE in patients with type 2 diabetic patients without structural heart disease.

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Materials and methods

Patient selection

A total of 210 patients with type 2 diabetes mellitus, who were admitted to Oita University Hospital from 1998 to 2004 for blood glucose control were recruited. Plasma NE was measured in the fasting and resting states in the supine position early in the morning. One hundred nineteen patients (68 men and 51 women) of 210 patients had plasma NE measured and confirmed retrospectively. Follow-up data were obtained in 95 patients (80%).

Type 2 diabetes mellitus was defined as a fasting plasma glucose concentration ≥126 mg/dl, a 2 h plasma glucose concentration following a 75 g oral glucose load ≥200 mg/dl, or the self-reported use of antidiabetic medication [13]. None of the patients had organic heart disease as determined by physical examinations, chest X-ray, 12-lead electrocardiogram (ECG), echocardiography, and ^{201}thallium SPECT. Myocardial ischemia was excluded by treadmill exercise ECG testing and ^{201}thallium SPECT. Essential hypertension was defined as diastolic blood pressure (BP) ≥90 mmHg, systolic BP ≥140 mmHg or self-reported use of antihypertensive medication [14]. Patients treated with alpha- or beta-adrenergic blocking agents, tricyclic antidepressants or other serotonin reuptake inhibitors, anti-platelet agents or with macroalbuminuria (≥500 mg/day), or abnormal plasma creatinine concentrations (≥1.2 mg/dl) were also excluded from the study. Dyslipidemia was defined as fasting triglycerides >200 mg/dl, or high-density lipoprotein (HDL) cholesterol ≤45 mg/dl in women and ≤35 mg/dl in men [15]. Hyperuricemia was defined as uric acid >6.0 mg/dl in women or >7.0 mg/dl in men [16]. Chronic kidney disease (CKD) was defined as an estimated glomerular filtration rate (e-GFR) <60 ml/min/1.73 m^2 [17]. Patients with an e-GFR <30 ml/min/1.73 m^2 were excluded. The hemoglobin (Hb) A1c value was determined by Japan Diabetes Society (JDS) method.

This investigation was conducted according to the principles expressed in the Declaration of Helsinki. Prior informed consent in terms of gathering BRS measurements was obtained from all patients, and the study protocol was approved by the institutional review board of Oita University.

Cardiac ^{123}I-MIBG scintigraphy

Planar studies were performed at 15 min (early) and 4 h (delayed) after the injection of 111 MBq of ^{123}I-MIBG using a rotating gamma camera equipped with a low-energy, parallel-hole, all-purpose collimator (ZLC 7500; Siemens, Munich, Germany). Data were analyzed with computer-based analysis software (SCINTIPAC; Shimadzu, Kyoto, Japan). The anterior planar images of the early and delayed ^{123}I-MIBG studies were analyzed visually. For semiquantitative analysis, the mean heart count per pixel (H) was calculated. Another region of interest was placed at a 10 mm × 10 mm area over the upper mediastinum, and the mean mediastinum count per pixel (M) was calculated. The H/M ratio was calculated from early and delayed anterior planar images [1,18]. After correcting for the physical decay of ^{123}I-MIBG, the percentage WR of the tracer from the myocardium was determined for the 4-h period. The WR was calculated from the initial and delayed images using the following formula without correction for the background and the time decay of ^{123}I label: $([(H)-(M)]\text{early} - [(H)-(M)]\text{delayed}) \times 100/[(H)-(M)]\text{early}$ (%), as previously reported [1,18]. In our hospital, the normal ranges of early H/M ratio, delayed H/M ratio, and WR were set at 2.0–2.7, 2.1–2.9, and 21–30%, respectively.

We defined enhanced WR and depressed delayed H/M ratio as ≥41.4% and ≤1.89, respectively, as we previously reported [19].

BRS measurements

For BRS assessment, all subjects were studied while in the supine position in a quiet room between 09:00 h and 11:00 h [1]. A catheter was inserted into the right cubital vein, and arterial blood pressure was recorded noninvasively using tonometry (Jentow-7700; Nihon Colin, Komaki, Japan) [1]. Arterial blood pressure and a 12-lead ECG were monitored simultaneously. Data were stored in a PCM data recorder (RD-200T; TEAC, Tokyo, Japan). After an interval of 30 min to allow the patient to stabilize, the patient was asked to breathe at a rate of 15 breaths/min using a metronome. BRS was assessed using the phenylephrine method [14]. Phenylephrine (2–3 μg/kg) was injected over 15 s to increase systolic blood pressure by 15–40 mmHg. BRS was calculated as the slope of the linear regression line relating the systolic blood pressure changes to the RR interval changes. Regression lines with more than 20 data points and a correlation coefficient (r) greater than 0.8 were accepted for analysis [6,14]. We defined depressed BRS as the value ≤5.63 ms/mmHg, as we previously reported [19].

Follow-up

Most of the follow-up for patients occurred at our hospital (Oita University Hospital). Information was obtained from those patients whose follow-up was performed by a general practitioner. Information on the patients who were hospitalized in other departments was also obtained. For the patients who died, the cause of death was documented with the help of the patients' family and general practitioners.

An end point was defined as the appearance of MACCE, which included cardiovascular mortality, nonfatal myocardial infarction, coronary revascularization through angioplasty or bypass, stroke, and congestive heart failure requiring admission. Using this combined criterion, only the first event was taken into account in statistical analysis.

Plasma NE cut-off values were determined from receiver operating characteristic curve (Fig. 1). The best plasma NE with the highest sensitivity and specificity to predict MACCE was 333 pg/ml. Therefore, we defined high NE patients as ≥333 pg/ml. From 1998,

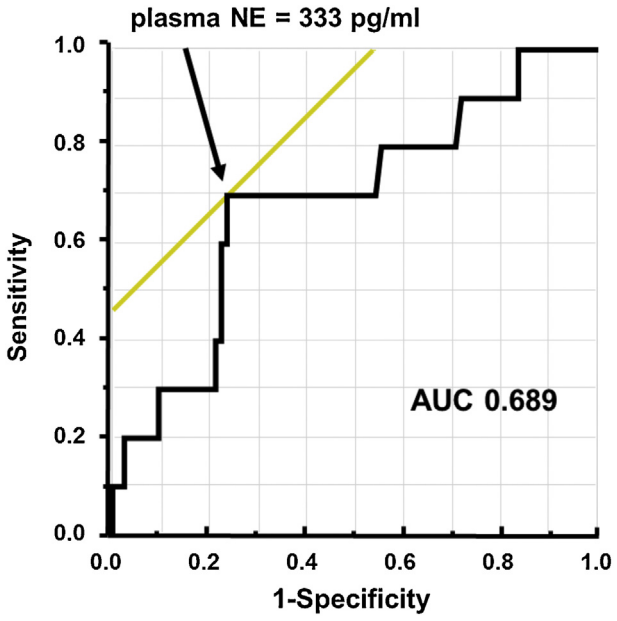

Fig. 1. Receiver-operating characteristic curve for the ability of resting plasma norepinephrine to predict major adverse cardiac cerebrovascular events. AUC, area under the curve.

accurate follow-up information for 3.6 ± 1.9 years was obtained in 27 high NE patients (13 female, mean age 64 ± 12 years) and 68 low NE patients (29 female, 60 ± 12 years).

Statistical analysis

Data are presented as mean ± SD. The chi-square test was used for categorical variables, and the analysis of variance (ANOVA) test was used for continuous variables. Kaplan–Meier MACCE-free analysis was used to compare MACCE-free times between the high NE group and the low NE group. Univariate and multivariate Cox proportional hazard regression analyses were performed to identify independent predictors (risk factors) of the MACCE. Risk factors entered into the risk model included age, gender, body mass index, smoking status, duration of diabetes, fasting plasma glucose, hemoglobin A1c, hypertension, dyslipidemia, hyperuricemia, e-GFR. Results are given as hazard ratios with 95% confidence intervals. A value of $p < 0.05$ was considered significant. Multivariate Cox regression analysis was performed only for variables with significant univariate impact. All computations were performed with JMP (JMP version 9.0.0, SAS, Cary, NC, USA), running under Windows 7 (Microsoft, Redmond, WA, USA).

Results

Patient characteristics

Baseline patient characteristics of high and low NE patients are presented in Table 1. The delayed *H*/*M* ratio ($p < 0.05$) and the resting plasma NE ($p < 0.0001$) were higher in the high NE patients than in the low NE patients. No significant difference was observed in terms of age, body mass index, duration of diabetes, fasting plasma glucose, or HbA1c, total cholesterol, triglyceride, high-density lipoprotein cholesterol, uric acid, creatinine, blood pressure, heart rate, or e-GFR. There was no difference between groups with regard to gender, current smoking, hypertension, dyslipidemia, or hyperuricemia. Regarding drug use, no significant differences between the 2 groups were observed in percentage of the patients who were being treated with angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, calcium channel blockers, beta blockers, or statins.

Table 1
Clinical characteristics of studied patients.

	High NE ($n = 27$)	Low NE ($n = 68$)	*p*-Value
Age (years)	64 ± 12	60 ± 12	NS
Gender (female/male)	13/14	29/39	NS
Body mass index (kg/m^2)	26 ± 6	24 ± 5	NS
Current smoker (%)	56	53	NS
Duration of diabetes (years)	9 ± 7	10 ± 9	NS
Fasting plasma glucose (mg/dl)	143 ± 47	160 ± 47	NS
Hemoglobin A1c (JDS) (%)	7.7 ± 1.5	8.3 ± 1.7	NS
Total cholesterol (mg/dl)	193 ± 34	204 ± 50	NS
Triglyceride (mg/dl)	134 ± 65	157 ± 183	NS
HDL cholesterol (mg/dl)	48 ± 16	46 ± 14	NS
Uric acid (mg/dl)	5.6 ± 1.7	5.3 ± 1.6	NS
Creatinine (mg/dl)	0.8 ± 0.3	0.8 ± 0.4	NS
Hypertension (%)	67	66	NS
Systolic BP (mmHg)	135 ± 19	129 ± 23	NS
Diastolic BP (mmHg)	71 ± 12	69 ± 14	NS
Heart rate (beats/min)	69 ± 9	70 ± 9	NS
Dyslipidemia (%)	81	75	NS
Hyperuricemia (%)	33	26	NS
e-GFR (ml/min/1.73 m^2)	77 ± 28	78 ± 29	NS
WR (%)	35 ± 10	40 ± 14	NS
Delayed *H*/*M* ratio	2.4 ± 0.7	2.1 ± 0.4	<0.05
BRS (ms/mmHg)	7.1 ± 4.9	7.0 ± 4.9	NS
Resting plasma NE (pg/ml)	427 ± 84	199 ± 68	<0.0001

Data are mean ± SD.
BP, blood pressure; BRS, baroreflex sensitivity; e-GFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; *H*/*M*, heart-to-mediastinum; JDS, Japan Diabetes Society; NE, norepinephrine; WR, washout ratio.

Patient outcomes

During the follow-up, 2 patients died (2%). Of the 2 deaths, one was cardiac death, while the other was attributed to non-cardiac causes. All causes of mortality were not significantly different between the high NE patients and the low NE patients. There were no significant differences in total cardiac mortalities between the 2 groups.

MACCE-free estimation

MACCE-free ratio as evaluated by Kaplan–Meier analysis was significantly lower in the high NE patients than in the low NE patients (log-rank 10.3, $p = 0.0013$) (Fig. 2).

Univariate and multivariate predictors of MACCE

During the follow-up, 10 patients presented with MACCE (11.0%). Of the 11 MACCEs, 1 was attributed to cardiac death, 0 were attributed to nonfatal myocardial infarctions, 1 was attributed to coronary revascularizations, 5 were attributed to strokes, and 3 were attributed to congestive heart failure (Table 2). The MACCEs were observed more frequently in the high NE patients (26%) than in the low NE patients (4%, $p < 0.005$). Results of univariate and multivariate Cox proportional hazards regression analysis of the MACCEs are presented in Table 3. The univariate analysis revealed that female gender, hyperuricemia, e-GFR, low BRS, and high NE were associated with the MACCE. On the following multivariate analysis using risk factors including female gender, body mass

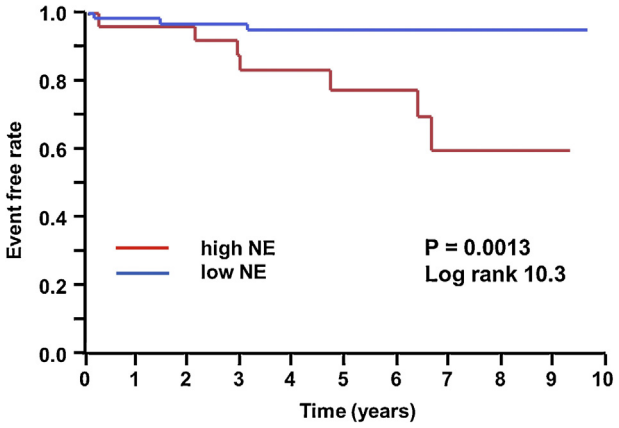

Fig. 2. Kaplan–Meier event-free curves for major adverse cardiac and cerebrovascular events between the high norepinephrine patients and the low norepinephrine patients. NE, norepinephrine.

Table 2
Number of patients developing MACCE.

	High NE ($n = 27$)	Low NE ($n = 68$)
Cardiac death	0	1
Nonfatal myocardial infarction	0	0
Coronary revascularization	1	0
Stroke	5	0
Congestive heart failure	2	1
Total	8	2

MACCE, major adverse cardiac and cerebrovascular events; NE, norepinephrine.

Table 3
Univariate and multivariate association of baseline characteristics with MACCE.

	Univariate *p*-Value	Multivariate Hazard ratio	95% CI	*p*-Value
Age (years)	0.5944			
Female gender	0.0154*	7.75	1.41–69.1	0.0172*
Body mass index (kg/m^2)	0.0240*	1.04	0.853–1.27	0.6968
Current smoker	0.1069			
Duration of diabetes (years)	0.9712			
Fasting plasma glucose (mg/dl)	0.8668			
Hemoglobin A1c (%)	0.6608			
Hypertension (%)	0.5000			
Dyslipidemia (%)	0.2194			
Hyperuricemia (%)	0.0052**	2.11	0.208–23.5	0.5243
e-GFR (ml/min/1.73 m^2)	0.0367*	0.98	0.937–1.01	0.2143
High WR (≥41.4%)	0.4775			
Low delayed *H*/*M* ratio (≤1.89)	0.1475			
Low BRS (≤5.63 ms/mmHg)	0.0047**	6.66	1.66–33.0	0.0077**
High NE (≥333 pg/ml)	0.0033**	5.40	1.21–29.3	0.0275*

BRS, baroreflex sensitivity; CI, confidence interval; e-GFR, estimated glomerular filtration rate; *H*/*M*, heart-to-mediastinum; MACCEs, major adverse cardiac and cerebrovascular events; NE, norepinephrine; WR, washout ratio.
* $p < 0.05$.
** $p < 0.01$.

index, hyperuricemia, e-GFR, low BRS, and high NE, female gender (hazard ratio 7.75, 95% confidence interval 1.41–69.1, $p = 0.0172$), low BRS (hazard ratio 6.66, 95% confidence interval 1.66–33.0, $p = 0.0077$), and high NE (hazard ratio 5.40, 95% confidence interval 1.21–29.3, $p = 0.0275$) independently predicted the incidence of MACCE by multivariate analysis (Table 3).

Discussion

In the present study, 95 Japanese type 2 diabetic patients were followed for a mean period of 3.6 years, and MACCE developed in 11 patients. The most important finding is that high NE value can accurately predict cardiovascular events in patients with type 2 diabetes without structural heart disease/severe complications, which is comparable to BRS value. MACCE-free ratio as evaluated by Kaplan–Meier analysis was significantly lower in the high NE patients than in the low NE patients.

In this study, cardiac sympathetic function was estimated by two different methods, including plasma NE concentration and ^{123}I-MIBG scintigraphy. The ^{123}I-MIBG is an analog of guanidine that shares the same neuronal transport and storage mechanisms with norepinephrine. In the heart, it is considered that reduced uptake of ^{123}I-MIBG (*H*/*M* ratio) reflects reduced norepinephrine content at presynaptic sits or reduced neural density, while an enhanced washout rate of ^{123}I-MIBG reflects enhanced release of norepinephrine from presynaptic sites [20]. We have previously reported in our series of investigations that cardiac ^{123}I-MIBG scintigraphic findings, such as *H*/*M* ratio and WR are useful to identify high-risk patients [9,19]. However, the current study revealed that resting plasma NE more accurately predicts MACCE than cardiac ^{123}I-MIBG scintigraphic findings. Although the reason is unclear, our results suggest that pathogenic sympathetic activation leading to MACCE may be identified by the assessment of resting plasma NE, more easily and less expensively compared to cardiac ^{123}I-MIBG scintigraphy. On the other hand, BRS with the phenylephrine method estimates reflex cardiovascular vagal activity. In this study, hazard ratio of low BRS predicting the incidence of the MACCE by multivariate analysis was greater than that of high NE. This result might suggest that the analysis of cardiovascular vagal activity rather than cardiac sympathetic function may be more sensitive in risk stratification in this population.

Benedict et al. demonstrated the importance of initial plasma NE levels as a powerful prognostic predictor for mortality and morbidity in patients with asymptomatic left ventricular (LV) dysfunction [10]. Elevation in plasma NE levels might contribute to the progression of the disease syndrome by impairing physiological vasodilation [21], adversely affecting cardiac loading through deleterious effects on myocardial function and structure [22], and inciting development of ventricular arrhythmias [23]. They explained that the mechanisms by which an increase in catecholamines might cause ischemic heart events were promotion of platelet aggregation and platelet thrombi formation [23], increased myocardial oxygen demand owing to an increase in heart rate, and predisposition to the development of ventricular arrhythmias [10]. The cut-off value of the plasma NE (393 pg/ml) in their study was higher than ours (333 pg/ml). This difference might be explained by the severity of the disease condition, i.e. ours included type 2 diabetic patients without severe complications while they included patients with LV dysfunction, and ischemic heart disease (about 90%).

On the other hand, in the Survival and Ventricular Enlargement (SAVE) Trial, only plasma renin activity and atrial natriuretic peptide were independently predictive of cardiovascular mortality, whereas the other neurohormones including plasma NE were not [12]. It should be further estimated as to which is the better predictor between plasma renin activity and plasma NE in our population.

Martin et al. observed that patients of apical ballooning syndrome had abnormal vasoreactivity and sympathetic responses to acute mental stress testing in the laboratory setting. There was a significant correlation between the percentage change in plasma NE levels and percentage decrease in endothelial function, as manifested by reactive hyperemia peripheral arterial tonometry score, after acute mental stress testing in all subjects [24]. Watanabe et al. demonstrated that endothelial function assessed by flow-mediated dilation was well correlated with sympathetic activity evaluated by the power ratio of low-frequency power to high-frequency power in the heart rate variability, and autonomic nervous system activity was an important regulatory factor of endothelial function in subjects with ischemic heart disease. The finding that this interaction was suppressed by beta-blockers and the presence of diabetes suggests that the autonomic nervous system had an effect on endothelial function [25]. Taken together, it is suggested that greater MACCEs in the high NE patients in this study might be associated with endothelial dysfunction.

Some evidence suggested that beneficial effects of beta-blockers on long-term survival of patients with chronic heart failure could be contributed to by heart rate reduction. It was strongly supported by the finding that improvement in survival was closely correlated with heart rate reduction but not with the dose of beta-blockers in heart failure treatment [26,27].

In this study, predictors of MACCE were not only low BRS and high NE, but also female gender. Huxley et al. explained why diabetes had a greater adverse effect in females than in males. According to their analysis, diabetes may induce a more unfavorable cardiovascular risk profile among females. They found that women with diabetes not only have significantly higher levels of blood pressure and lipids than males with diabetes but also that the difference in the levels among people with and without diabetes was significant greater in females than it was in males [28]. We previously demonstrated that depressed BRS value can accurately predict cardiac cerebrovascular events, especially in female patients with type 2 diabetes. Once diabetic, a female who develops an impaired BRS has a worse prognosis than a similar male [29].

In this study, it was not clear if the mechanism of the elevated plasma NE in the high NE patients was associated with autonomic dysfunction in diabetic patients. At least no significant difference was observed in terms of age, body mass index, duration of diabetes, or diabetic control between the high NE patients and the low NE patients. Although the patients with the organic heart disease as determined by physical examinations were excluded, we did not evaluate the detailed cardiac diastolic function derived from septal peak left ventricular relaxation velocity by tissue Doppler. Cardiac diastolic dysfunction was suggested to be the cause of elevated plasma NE.

Several limitations should be considered in the present study. First, the average age of high NE patients was not matched with that of low NE patients. Second, clinical manifestations of diabetic autonomic neuropathy involve many categories, including cardiovascular, gastrointestinal, genitourinary, metabolic, sudomotor, and papillary. Because we did not quantitatively evaluate other autonomic neuropathy, the association between plasma NE and other systemic autonomic neuropathy remains unclear. Third, we could not precisely evaluate serial changes of diabetic status including plasma glucose level and HbA1c during follow-up. Finally, in the previous study, the patients with mild heart failure, whose resting plasma NE levels were only mildly elevated, exercise plasma concentrations could provide prognostic information for cardiac death [30]. We did not evaluate exercise plasma NE concentrations in this study.

Conclusion

Our results suggest that resting plasma NE is comparably useful to identify high-risk patients for MACCE to BRS in type 2 diabetic patients. The results also suggest that pathogenic sympathetic activation leading to MACCE may be identified by the assessment of resting plasma NE, more easily and less expensively compared to cardiac ^{123}I-MIBG scintigraphy in this population.

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Authors' contribution

Kunio Yufu analyzed data and wrote and revised/edited the manuscript; Kunio Yufu, Norihiro Okada, and Yukichi Murozono provided patient care and samples, collected data, compiled database; Yuki Ebata, Tetsuji Shinohara, and Mikiko Nakagawa supervised the overall study with Naohiko Takahashi, provided patient care and samples, and revised the manuscript.

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